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FIG. 1 - AoPRT-L cDNA sequence

```
          10        20        30        40        50        60        70
          |         |         |         |         |         |         |
ATGGCTCTATCCAAAGCTTTCACCTCCCTCCTCCTCCTCCCTGTCCTCCTCCTGCCCCTCGCCTCCGCCGCCACCTTC
M  A  L  S  K  A  F  T  S  L  L  L  L  P  V  L  L  L  P  L  A  S  A  A  T  F

 80        90        100       110       120       130       140       150
 |         |         |         |         |         |         |         |
  < P1 ccaacaaatgcacctacaccgaattccgcg                          cgcctgcagccaa
ACCGTCACCAACAAATGCACCTACACCGTCTGGGCCGCTGCAGTGCCGGGGGGCGGTCGCCGCCTCGACCCCAACCAA
T  V  T  N  K  C  T  Y  T  V  W  A  A  A  V  P  G  G  G  R  R  L  D  P  N  Q

   160       170       180       190       200       210       220       230
   |         |         |         |         |         |         |         |
tcctggaccctcaccg  >P2
TCCTGGACCCTCACCGTCGCCCCCGGTACCACCGGTGCCCGCATCTGGGGCCGAACCGGCTGCTCCTTCGACCCCTCT
S  W  T  L  T  V  A  P  G  T  T  G  A  R  I  W  G  R  T  G  C  S  F  D  P  S

     240       250       260       270       280       290       300       310
     |         |         |         |         |         |         |         |
GGCCACGGCCATTGCCAGACCGGTGACTGCGGCGGTCTCCTTGCCTGCACCGCCTACGGCTCCCCTCCCGACACCCTC
G  H  G  H  C  Q  T  G  D  C  G  G  L  L  A  C  T  A  Y  G  S  P  P  D  T  L
```

```
         320       330       340       350       360       370       380       390
           |         |         |         |         |         |         |         |
GCAGAATTCGCCCTGAACCAGTACGCCGGCCAGGACTTCTACGACATCTCCCTCGTCGACGGCTTCAACATCCCCATG
A  E  F  A  L  N  Q  Y  A  G  Q  D  F  Y  D  I  S  L  V  D  G  F  N  I  P  M

           400       410       420       430       440       450       460
             |         |         |         |         |         |         |
GACTTCTCCCCGACGTCCGGAAATTGCCACGACATCCGGTGCACCGCGGACATCAACGGTCAGTGCCCGGCGGAGCTG
D  F  S  P  T  S  G  N  C  H  D  I  R  C  T  A  D  I  N  G  Q  C  P  A  E  L

 470       480       490       500       510       520       530       540
   |         |         |         |         |         |         |         |
AAGGCACCCGGGGGGTGTAACAACCCGTGCACCGTGTTCAAGACCAATGAGTACTGCTGCACTTCGGGAGGCTGTGGG
K  A  P  G  G  C  N  N  P  C  T  V  F  K  T  N  E  Y  C  C  T  S  G  G  C  G

   550       560       570       580       590       600       610       620
     |         |         |         |         |         |         |         |
CCCACGGACTATTCCAAGTTTTTCAAGCAGAGGTGCCCTGATGCGTACAGTTACCCCAAGGATGACGCTACCAGCACT
P  T  D  Y  S  K  F  F  K  Q  R  C  P  D  A  Y  S  Y  P  K  D  D  A  T  S  T

     630       640       650       660       670       680       690       700
       |         |         |         |         |         |         |         |
TTTACTTGTCCCAGTGGGGCTGATTACAGGGTTGTGTTCTGCCCTTGATCGAGCTTACTCAGATGTTGTGTGAGCAAT
F  T  C  P  S  G  A  D  Y  R  V  V  F  C  P  *

       710       720       730       740       750       760       770
         |         |         |         |         |         |         |
CAAACTATGGTTAATTTGTACGTAGCTCATTAAGAACGGAATAAGGTCGCATGTAAGCTCTACTTGAGC
```

FIG. 1 CONT'D

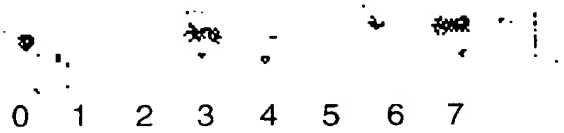
FIG. 2

Similarity of AoPRT-L to other PR-5 Group Proteins

Protein	Cellular Location	pI	Percentage Similarity to AoPRT-L	Percentage Identity to AoPRT-L
AoPRT-L	Extracellular	4.9	100	100
Osmotin	Vacuolar	7.5	89	77
Tobacco Osmotin-like	Vacuolar	7.5	89	77
Tobacco Thaumatin-like	Extracellular	5.2	80	80
Tomato NP24	Vacuolar	7.8	78	65
Thaumatin	Cytoplasmic	12.0	76	63
Potato Osmotin-like	?	6.1	76	62
Rice Thaumatin-like	?	5.0	70	53
Wheat Thaumatin-like	Extracellular	4.5	68	49
Barley Thaumatin-like	Extracellular	4.2	67	49

FIG.3a

Induction of AoPRT-L following cell isolation



Time after isolation (days)

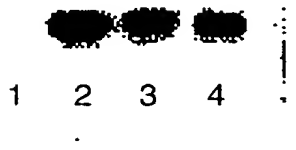
FIG.3b

Induction in etiolated seedlings by wounding

Time after wounding (days)
Explant length 5mm

FIG.3c

Induction of AoPRT-L in whole plants by SA



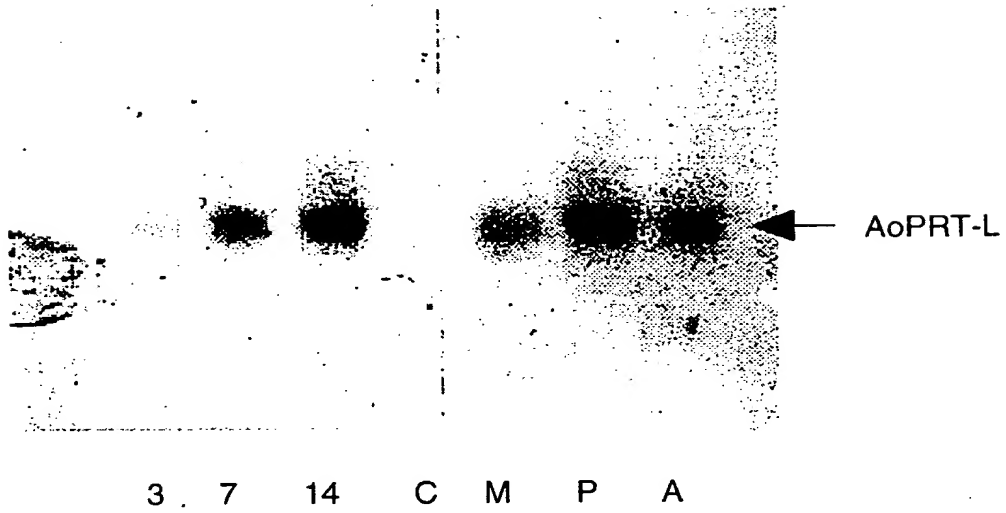
1; Water treated
2; 3 days after foliar spraying with 1mM SA
3; 3 days after continuous root feeding with 1mM SA
4; 3 days after initial root feeding with 1mM SA

Time course of induction following foliar application of 1mM SA to whole plants

Time after application (days)

FIG.4

AoPRT-L Expression in Asparagus seedlings infected with *Stemphyllium versicarium*



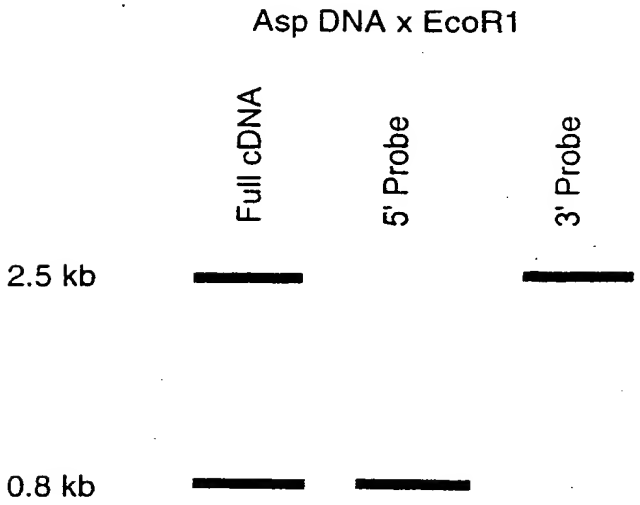
Figures (3, 7 & 14) indicate days after symptom development

C - uninfected Asparagus
M - Infected region (day 14)
P - Pigmented region (day 14)
A - Asymptomatic region (day 14)

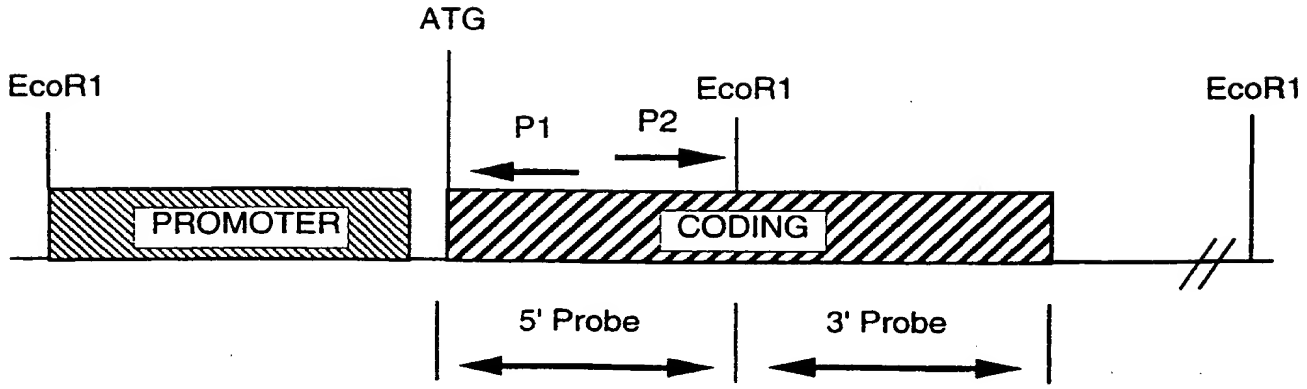
FIG. 5

IPCR Strategy

Southern Analysis



Primer Design



AoPRT-L Promoter sequence : Similarities with other Defence Genes

```
-472 GAATTCTTAT TGCGACCTGA CTCTCTTGTT GTGCTGCCGA GGTGCTGTCG
-422 AAATTTCTGT TGCGCACAAC ATACTGGTCC TTGCTTGATT TGACAGTTCC
-372 AATAATTATT TCCATGTCAT GAGAGAAGCA CATGACTAAA GTAATTAGCT
-322 TAATCCCCTA AAACTCAATA CAAACGAGAT GACACATCCA CAGAAAAAAT
-272 TCTAATTAGT CTTTGCGTGT AGAAATTGGA AACTGAATAC CTACATTAAT
-222 TACAACTTTT GCAAATAAAA TATAAAGAAA GTTCTAACAT GAAGACTAGT
-172 TCTAACATGA AGACTAGTCC ACGAACTCGT ACCTTATTCC ACAAAGGCTT
-122 AGACTTTCCA CAAATCGAGA TTATCCCATG GACTGATGGA CACCATCCAA
-72  ATTATCCCTA TAAATACCTG CCCATTCCCC TCCTCCAGAC TCATCTAACT
-22  CAAAAACAAC ACACAACCAA TCATG
```

FIG. 6

Potato Wound-Induced

Tobacco PR-2

Carrot PR-3 and PR-4

18 bp repeat

TATA Box

pIPCR-TA

PCR using 5' and 3' primers
Clone into pJIT60 using KpnI and PstI

↓

p22-JIT60
Clone in GUS(INT) using BamHI and EcoRI

↓

p22-GUS(INT) JIT60

Cut with KpnI and XhoI and clone into KpnI and SalI cut pBin19

↓

p22-GUS(INT) Bin19

FIG. 7

FIG.8

Histochemical localistion of GUS activity in untreated stems from transgenic tobacco harbouring AoPRT-L-GUS or PR-1a-GUS

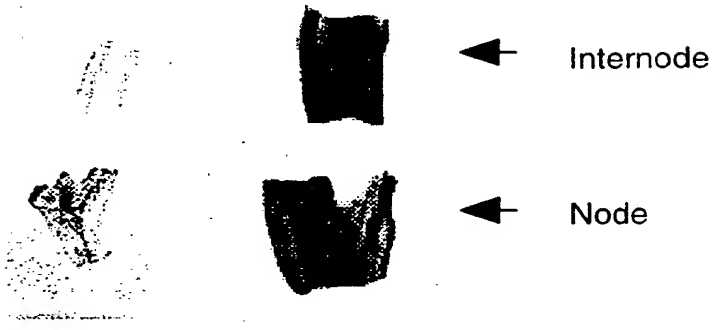
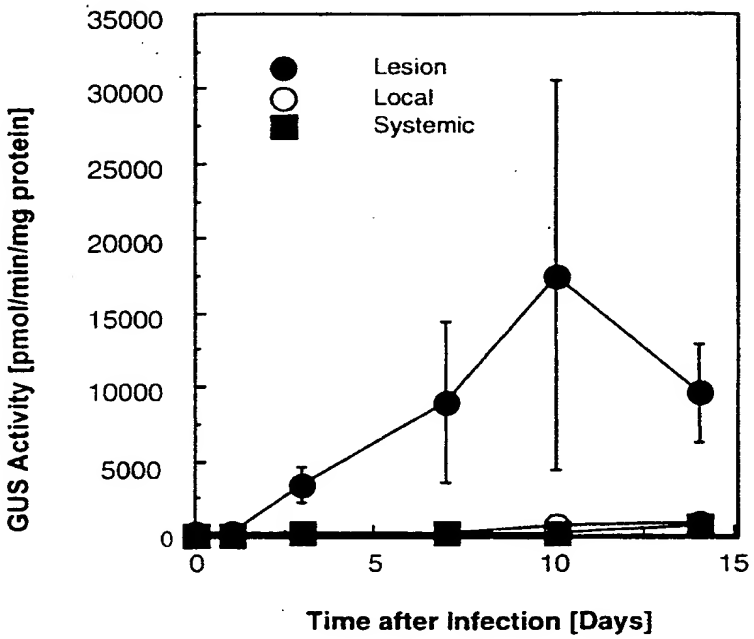
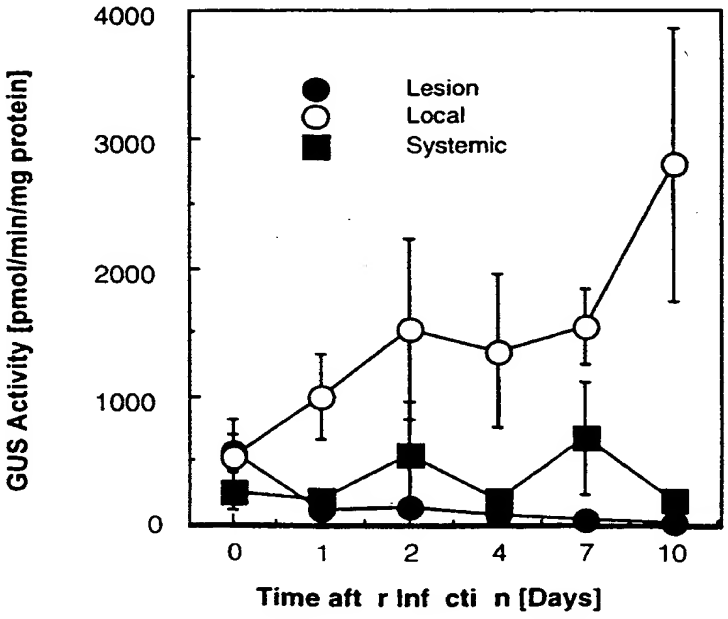


FIG. 9

AoPRT-L-GUS Expression in TMV-infected Tobacco



AoPRT-L-GUS Expression in Tobacco infected with *Pseudomonas syringae* pathovar *phaseolicola*



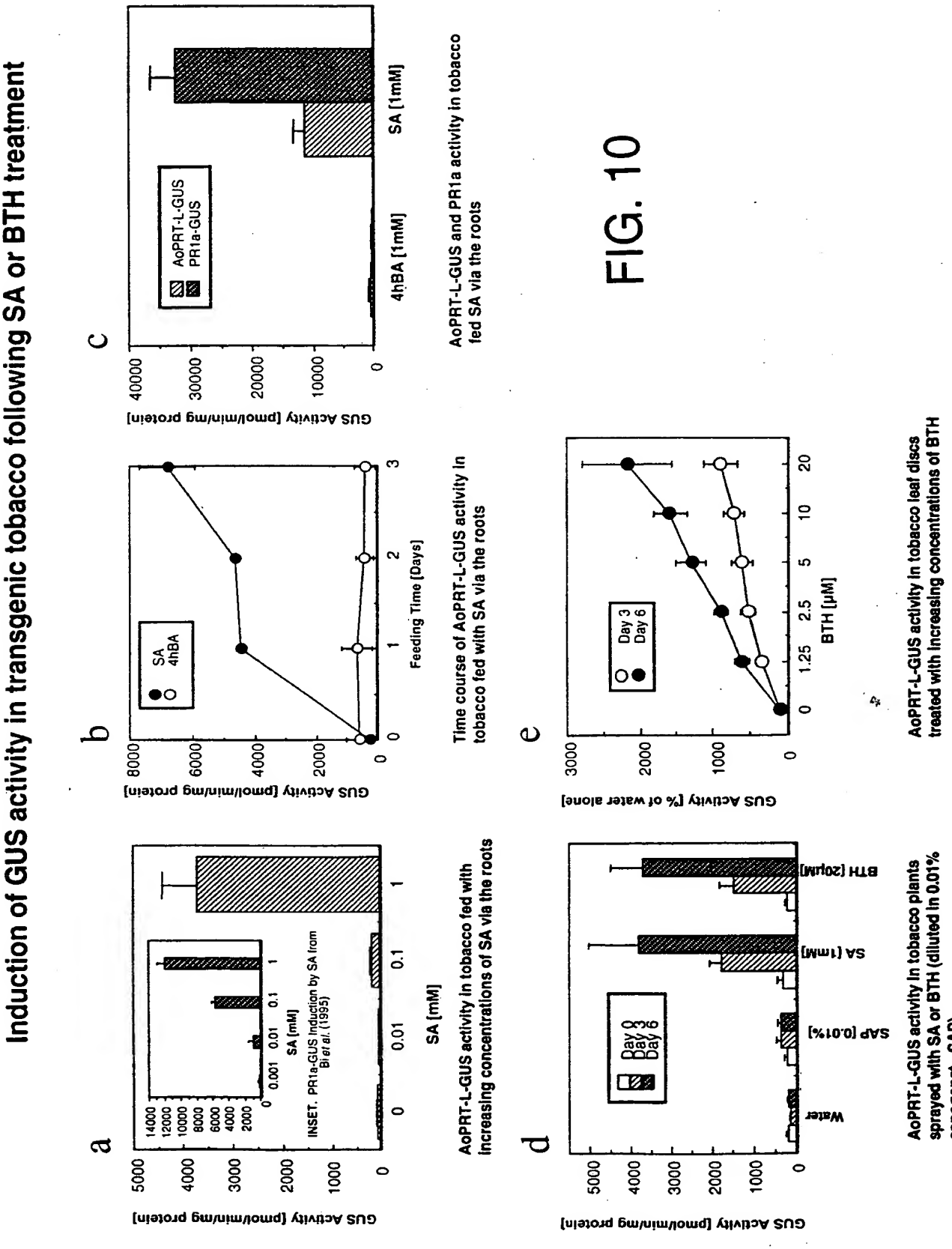
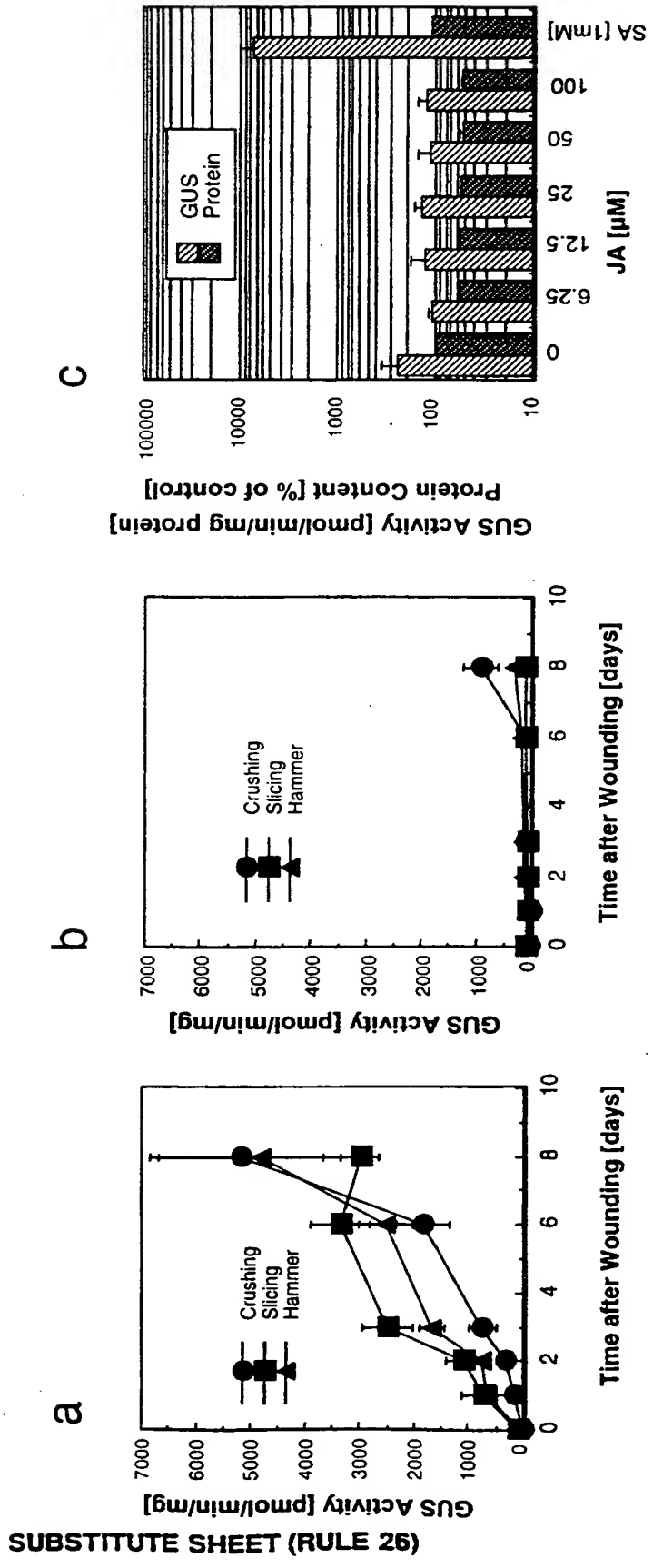

Induction of GUS activity in transgenic tobacco following SA or BTH treatment
a
GUS Activity [pmol/min/mg protein]
SA [mM]
INSET. PR1a-GUS Induction by SA from Bi et al. (1995)
AoPRT-L-GUS activity in tobacco fed with increasing concentrations of SA via the roots
b
SA
4hBA
Feeding Time [Days]
Time course of AoPRT-L-GUS activity in tobacco fed with SA via the roots
c
AoPRT-L-GUS
PR1a-GUS
4hBA [1mM]
SA [1mM]
AoPRT-L-GUS and PR1a activity in tobacco fed SA via the roots
d
Day 3
Day 6
Water
SAP [0.01%]
SA [1mM]
BTH [20µM]
AoPRT-L-GUS activity in tobacco plants sprayed with SA or BTH (diluted in 0.01% sapogenat - SAP)
e
Day 3
Day 6
BTH [µM]
GUS Activity [% of water alone]
AoPRT-L-GUS activity in tobacco leaf discs treated with increasing concentrations of BTH

FIG. 10

FIG. 11

Effects of wounding and JA on GUS expression in transgenic plants



AoPRT-L-GUS Expression Following Water Stress

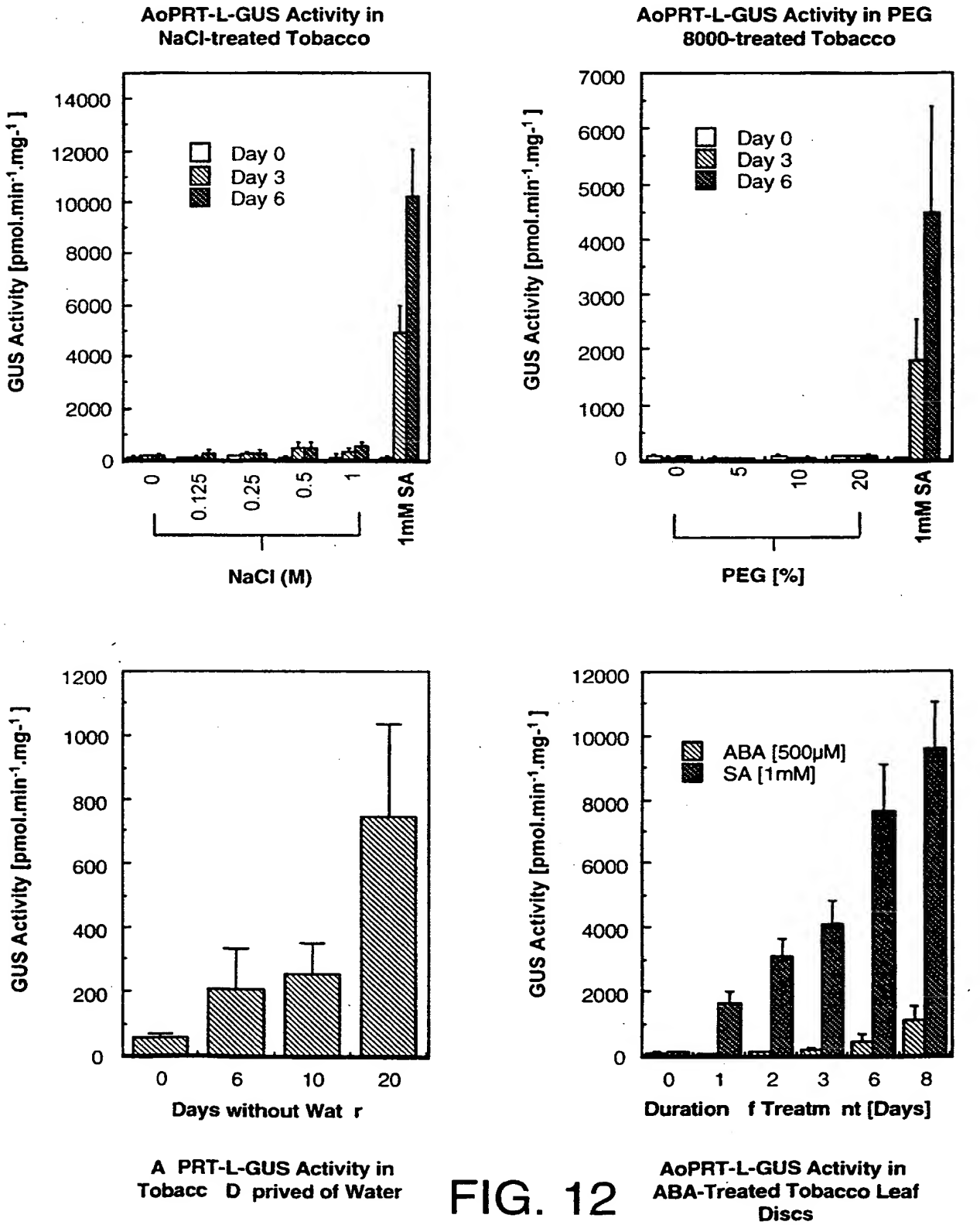


FIG. 12

FIG. 13 AoPRT-L-GUS Expression following Oxidative Stress

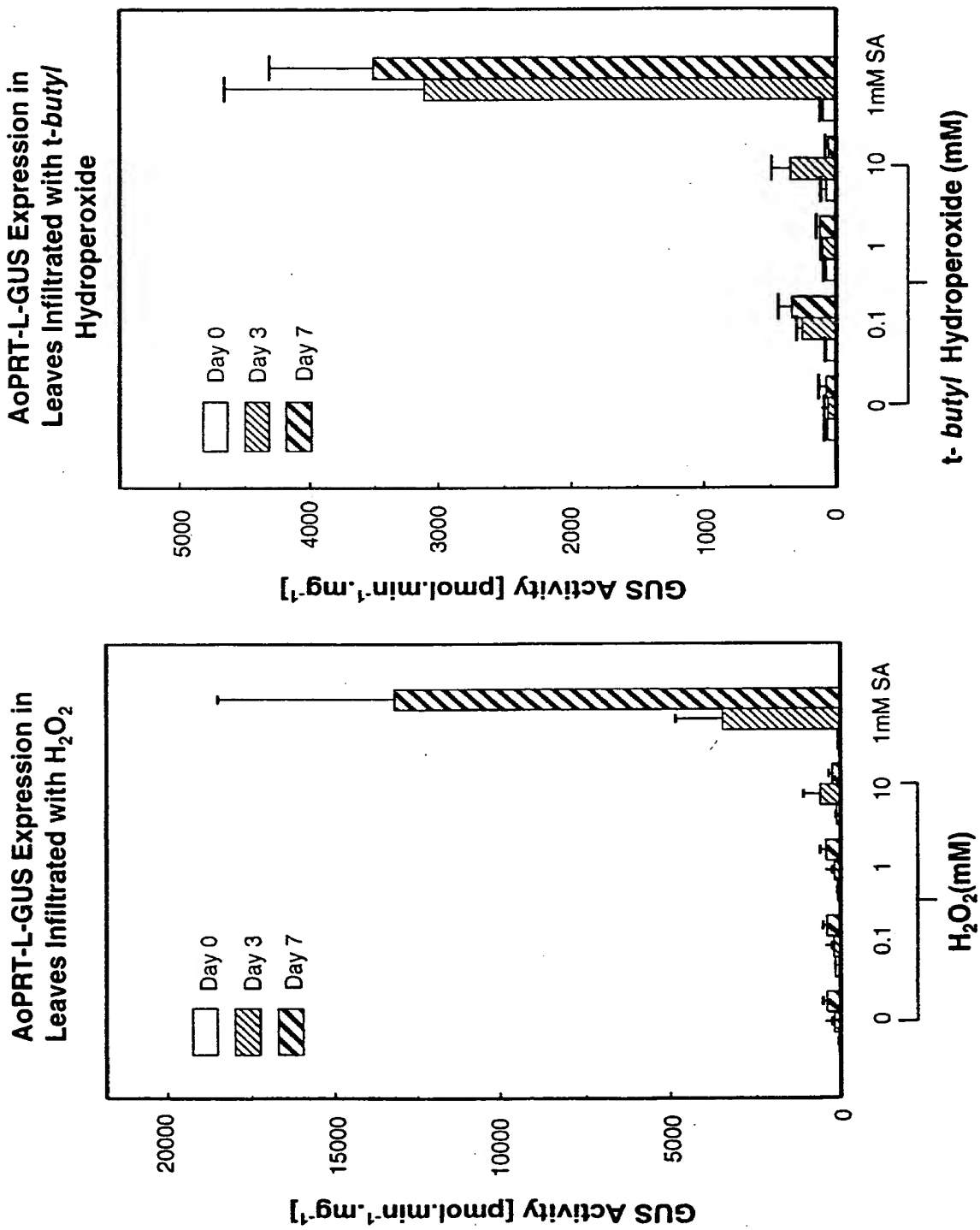


FIG. 14

AoPRT-L-GUS and Pr1a-Gus expression after SA or BTH induction in Brassica napus leaves

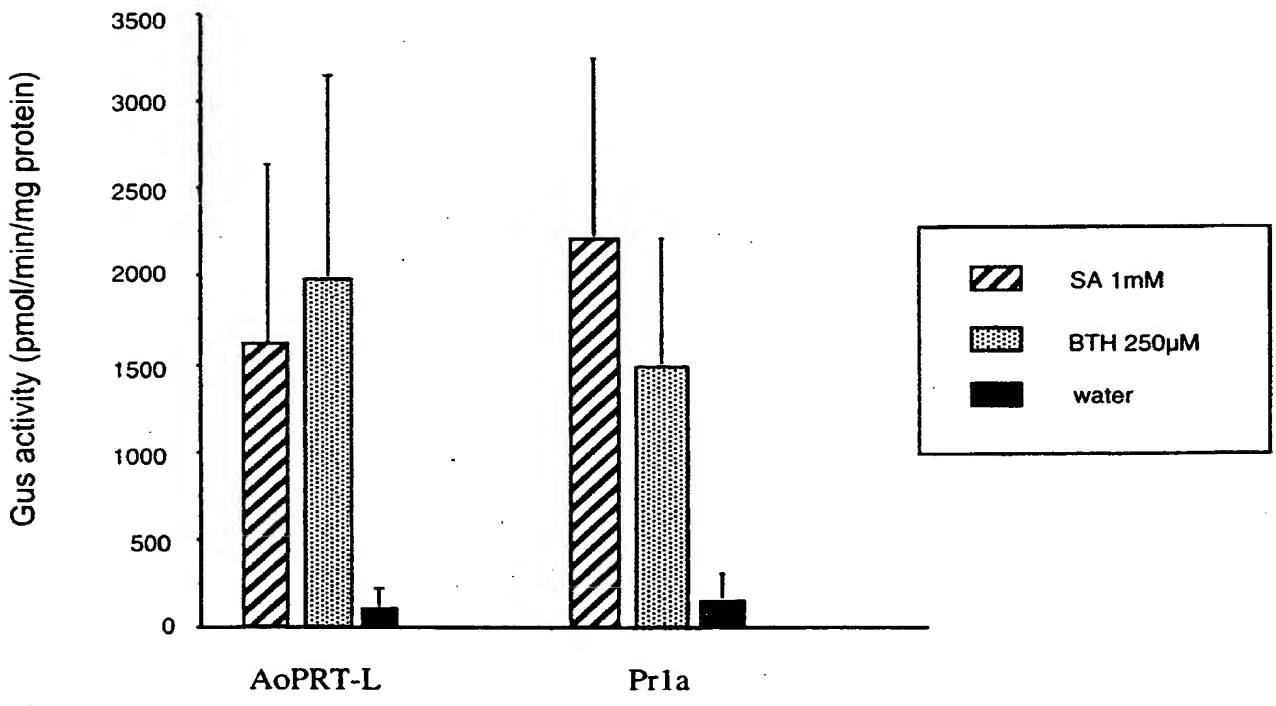


FIG. 15a **AoPRT-L promoter deletions**

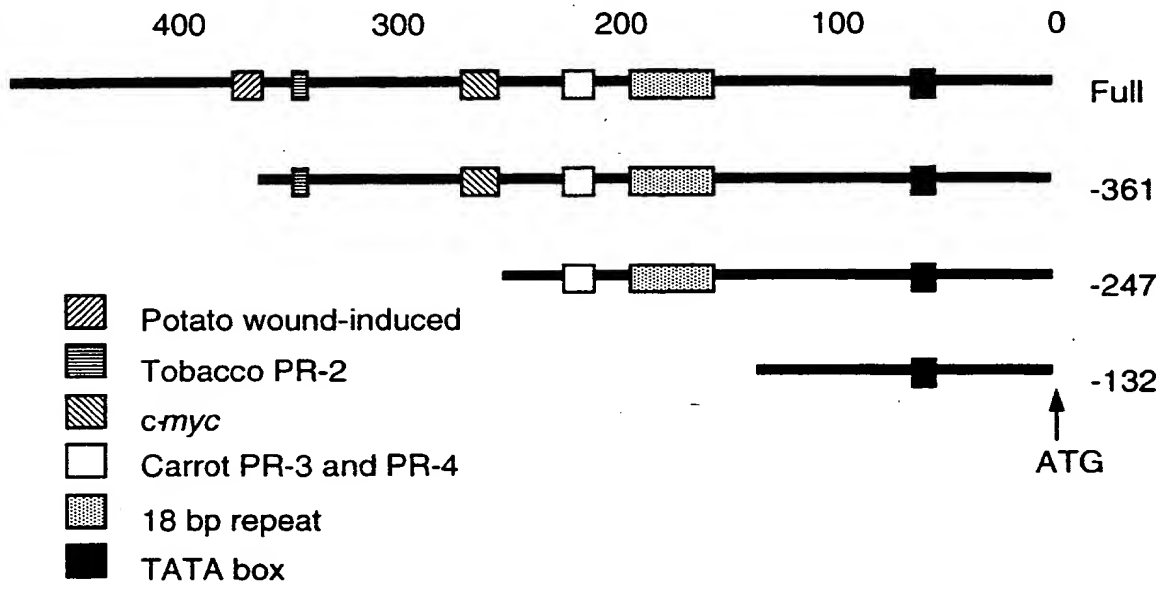
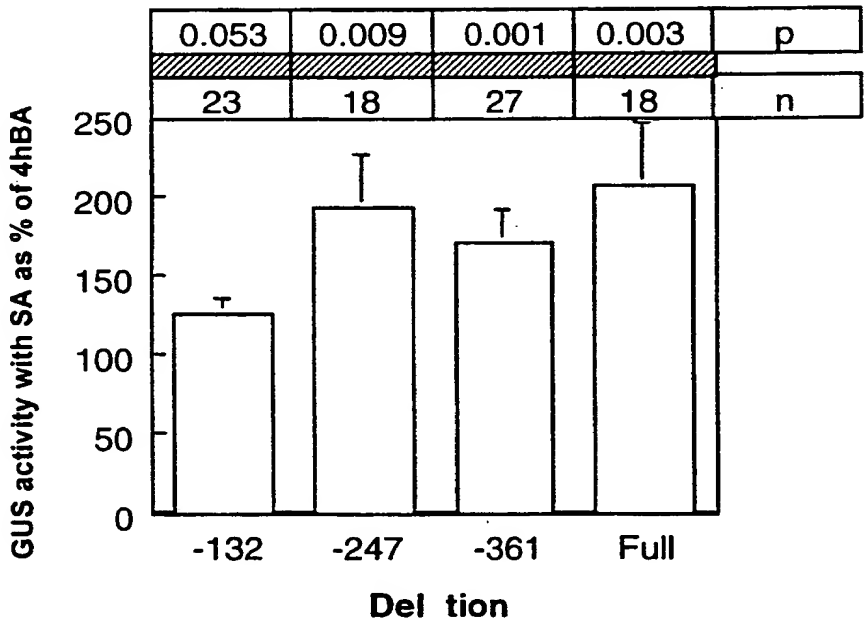


FIG. 15b **SA-responsiveness of AoPRT-L promoter deletion-GUS constructs in T0 transgenic tobacco plants**



n - number of individual transformants

p - probability that activity with SA is not different to activity with control-treatment (Wilcoxon signed rank test)

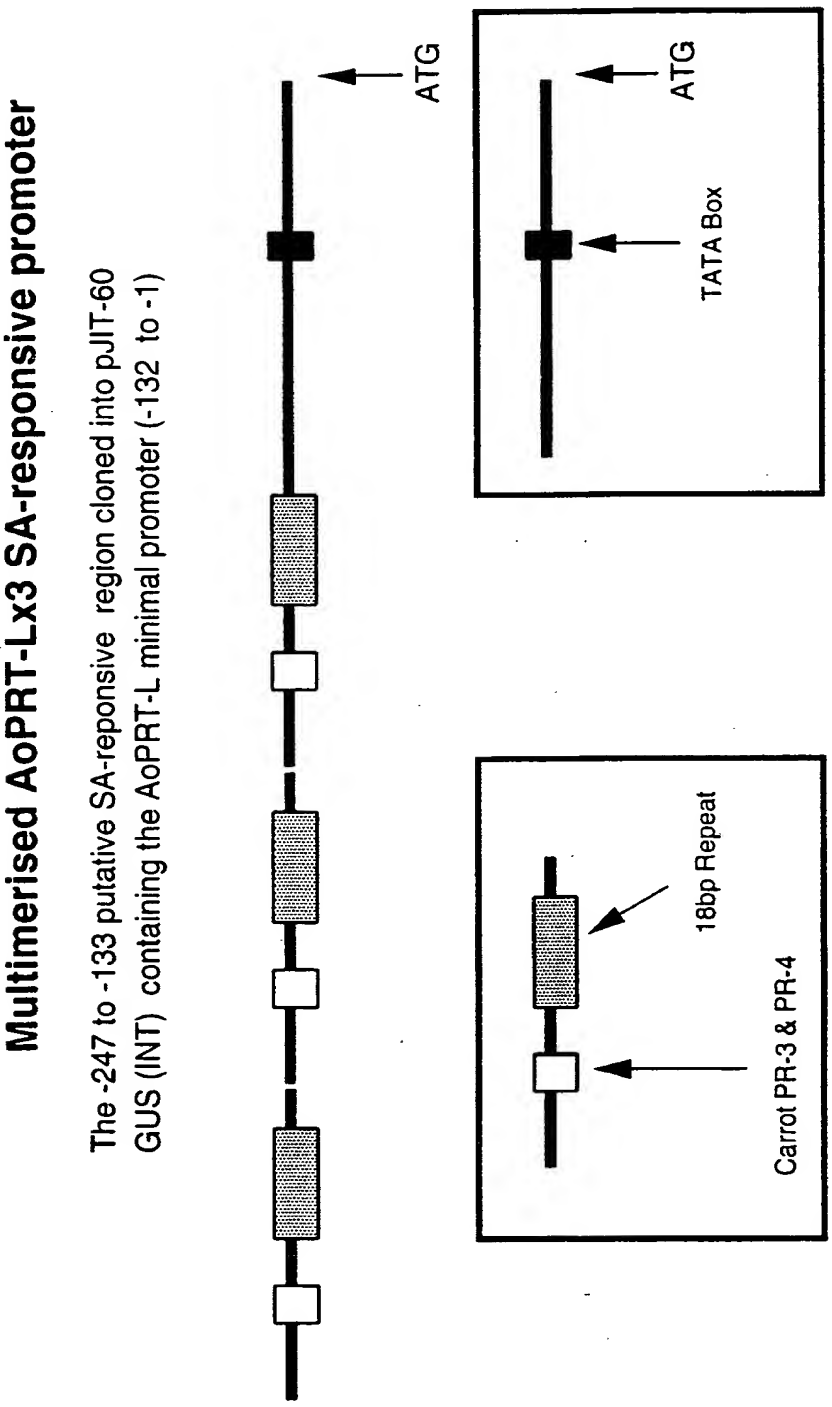

Multimerised AoPRT-Lx3 SA-responsive promoter
The -247 to -133 putative SA-reponsive region cloned into pJIT-60
GUS (INT) containing the AoPRT-L minimal promoter (-132 to -1)
ATG
ATG
TATA Box
AoPRT-L-min
(-132 to -1)
18bp Repeat
Carrot PR-3 & PR-4
AoPRT-L-SA
(-247 to -133)

FIG. 15c

FIG. 16

schematic diagram of plasmid pGB24